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Optimal Parameters for Laser Tissue Soldering. Part I: Tensile Strength and Scanning Electron Microscopy Analysis

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Background and Objectives: **The use of liquid and solid albumin protein solders to enhance laser tissue repairs has been shown to significantly improve postoperative results. The published results of laser-solder tissue repair studies have, however, indicated inconsistent success rates. This can be attributed to variations in laser irradiance, exposure time, solder composition, chromophore type, and concentration. An in vitro study was performed using indocyanine green-doped albumin protein solders in conjunction with an 808 nm diode laser to determine optimal laser and solder parameters for tissue repair in terms of tensile strength and stability during hydration.**
Study Design/Materials and Methods: **Twenty-five different combinations of laser irradiance (6.4, 12.7, 19.1, 25.5, 31.8 W/cm^2) and exposure time (20, 30, 40, 50, 100 or 40, 60, 80, 100, 200 seconds) were used. The effect of changing bovine serum albumin (BSA) concentration (25% and 60%) and indocyanine green (ICG) dye concentration (2.5 mg/ml and 0.25 mg/ml) of the protein solder on the tensile strength of the resulting bonds was investigated. The effect of hydration on bond stability was also investigated using both tensile strength and scanning electron microscopy analysis.**
Results: **Tensile strength was observed to decrease significantly with increasing irradiance. An optimum exposure time was found to exist where further irradiation did not improve the tensile strength of the bond. Tensile strength was found to be**

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greatly improved by increasing the BSA concentration. Finally, the lower ICG dye concentration increased the penetration depth of the laser light in the protein solder leading to higher tensile strengths. The strongest repairs were formed by using 6.4 W/cm² irradiation for 50 seconds with a protein solder composed of 60% BSA and 0.25mg/ml ICG. In addition, the solid protein solder provided more stable adhesion to the tissue than did the liquid protein solder when the tissue was submerged in a hydrated environment.
Conclusions: **This study greatly enhances the current understanding of the various factors affecting the soldering process. It provides a strong basis for optimization of the laser light delivery parameters and the solder constituents to achieve strong and reliable laser tissue repairs. Lasers Surg. Med. 24:319–331, 1999.** **© 1999 Wiley-Liss, Inc.**

Key words: diode laser; indocyanine green dye; protein solder; tissue repair

INTRODUCTION

Tissue heating by laser irradiation is used in many medical and surgical applications. In direct laser anastomosis of tissues, proteins within the target tissue are coagulated to form a bond between the two adjoining edges [1,2]. Compared with conventional microsuturing this technique offers reduced suture and needle trauma [3], reduced foreign body reaction [4], reduced bleeding [5], the potential to form an immediate watertight anastomosis intraoperatively [6], and shorter operating times [7].

Although some success has been achieved in experimental applications, two disadvantages of the laser-assisted procedure are foreseen for the clinical application of the technique. The first is low strength of the resulting anastomosis especially in the acute healing phase up to 5 days postoperative [8,9], and the second is thermal damage of tissue by direct laser heating [10–12]. Technical difficulties with tissue alignment [6], an ambiguous indication of the "endpoint" for the procedure [6], and poor reproducibility [13] are also concerns. Two advances have been useful in reducing the problems of low strength and thermal damage associated with laser tissue welding: (i) the addition of endogenous and exogenous materials to be used as solders and (ii) the application of laser wavelength-specific chromophores. The combination of serum albumin and indocyanine green (ICG) dye with an ~800 nm diode laser, first described by Bass et al. [14,15] has been favored by researchers. The light sensitive ingredient in the protein solder is the ICG dye which has a maximum absorption coefficient at 805 nm of 2×10^5 $M^{-1}cm^{-1}$ [16]. As tissue absorption at this laser wavelength is low, the dyed solder absorbs most of the incident light energy. Consequently, the amount of collateral tissue thermal damage due to direct absorption of the laser light is minimized. The increased temperature at the repair site during laser treatment denatures the albumin solder, which enhances the adhesion of the adjacent tissue edges. The resulting solder coagulum is non-immunogenic [17] and is gradually absorbed in the normal wound healing process [18,19]. Moreover, the presence of the solder coagulum increases the initial strength of the repair [6].

The efficacy of laser tissue soldering using ICG-doped albumin protein solders has been demonstrated in a range of tissues including the gastrointestinal tract [20,21], blood vessels [22–24], trachea [25], nerves [18,19,26–28], bladder [29], urethra [30–34], skin [35–37], dura mater [38], and cartilage [37]. The laser solder repair technique has been reported in many of these studies to be much quicker to perform than conventional suture techniques, as well as providing an immediate leak-free closure, with improved histological behavior. However, the reported acute tensile strength of the repairs has not been as high as their sutured opponents.

Lauto et al. have shown that the strength of solder-tissue bonds are dependent on protein concentration [18,19,28]. Bovine serum albumin (BSA) concentrations of 25, 50, and 66% (2.5 mg/ml ICG) formed bonds having acute tensile strengths of 10.4, 37.5, and 69.2%, respectively, of that of sutured nerves. Chan et al. investigated the effects of hydration on laser tissue solder repairs by using scanning electron microscopy analysis [37,39]. The solder was observed to detach from the tissue substrate once the specimens

were submerged in a hydrated environment of phosphate-buffered aqueous saline. This was traced to inadequate heat transmission through the solder, which produced unstable protein globules, rather than a homogeneous solder coagulum. In a subsequent study [40,41] it was found that the tensile strength of repairs could be improved by an order of magnitude (257 N/cm^2 compared with 5.2 N/cm^2 [6], 31 N/cm^2 [30], and 2.8 N/cm^2 [17]) if a series of small (0.2 ml) droplets of solder (25% BSA and 2.5 mg/ml ICG) were applied to the tissue, and each droplet was coagulated individually.

Despite the use of equivalent laser systems and solder components, a wide variation in laser irradiance, exposure time, solder composition, chromophore, and chromophore concentration were reported in these published results. The optimal parameters for laser tissue soldering were not determined. This in vitro study was performed to investigate the relative importance of these parameters to laser tissue soldering. An 808 nm diode laser was used in conjunction with an ICG-doped albumin protein solder to repair bovine aorta specimens. Liquid and solid protein solders prepared from 25% and 60% BSA, respectively, were compared. The effects of laser irradiance and exposure time on tensile strength of the repair as well as the effect of hydration on bond stability were investigated. In addition, two ICG dye concentrations, 2.5 mg/ml and 0.25 mg/ml, were used in each case to give light penetration depths in the protein solder of about 35 μm and 85 μm, respectively.

This article is the first of a two part study. It reports comparative results between liquid and solid solders prepared from a premix of ICG dye and protein. Part II of this study reports results from experiments carried out using premixed dye/solders vs. the use of solders with separate application of the dye.

MATERIALS AND METHODS

Protein Solder Preparation

Liquid protein solder solution was prepared from BSA (Sigma Chemical Co., St. Louis, MO) (25%) and ICG dye (2.5 mg/ml and 0.25 mg/ml) (Sigma Chemical Co., St. Louis, MO), mixed in deionized water. The protein solder was stored in a light-proof plastic vial in a refrigerator until required. Solution remaining after 10 days was discarded. Solid protein solder strips were prepared from BSA (60%) and ICG dye (2.5 mg/ml and 0.25 mg/ml) mixed in deionized water. The mixture was pressed to a thickness of 0.15 ± 0.01 mm and then cut into rectangular strips having nominal dimensions of 3 × 1 mm and allowed to dry. The solid protein solder strips were stored in a light proof container between two inert metal plates in a refrigerator until required. Strips not used within 10 days were discarded. Before use, the protein solders were allowed to reach room temperature.

Tissue Preparation

Bovine thoracic aortas were obtained from a slaughter house in Texas (Taylor Meat Company, Taylor, TX). The aortas were rinsed with phosphate-buffered saline, wrapped in saline-soaked gauze and stored at −70°C [37] until required. Before use, aortas were thawed and cut into rectangular specimens having approximate dimensions of 2 × 1 cm. The excess adventitia and media was trimmed to obtain a specimen thickness of approximately 1 mm.

Surgical Procedure

A full thickness incision was cut through the specimen width using a scalpel and the severed ends were placed together. Laser soldering was performed on the intima side of the aorta in both cases. Use of the liquid protein solder involved the deposition of 2 μl of protein solder perpendicularly across the junction of the severed aorta specimen using a micropipette (Hamilton Company, Reno, NV). The protein solder was spread to have approximate surface dimensions of 3 × 1 mm to match that of the solid protein solder. The solder was denatured with a continuous pass of the diode laser output. This is portrayed schematically in Figure 1. Use of the solid protein solder involved the placement of a protein solder strip perpendicularly across the junction of the severed aorta specimen. The solder was denatured with a continuous pass of the diode laser output. This is portrayed schematically in Figure 1. The exposure time required for the liquid protein solder to coagulate was about twice the optimum time required for the solid protein solder repairs. This was most likely due to latent heat required during vaporization of water in the liquid protein solder.

The investigation was divided into two parts during which a total of 1,440 bovine aorta tissue repairs were performed in vitro and tested.

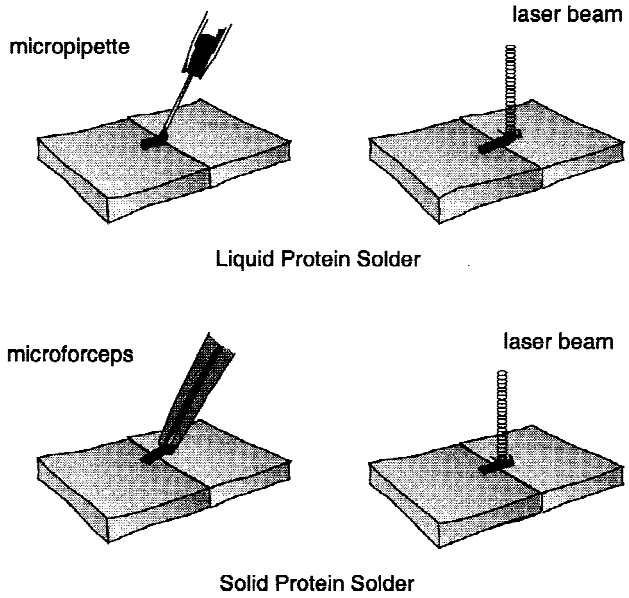


Fig. 1. Schematic of the laser tissue soldering procedure. ICG-doped albumin protein solder strips were positioned perpendicularly across the junction of the severed ends of the tissue and denatured with a continuous pass of the output from an 808nm diode laser.

Laser System

A GaAlAs semiconductor laser diode with a nominal output power of 1.5 W and wavelength of 808 nm (Endodiode 10000, Alcon Surgical Inc., Irvine, CA) was used to denature the protein solder. Two modes of light delivery were used:

In the first part of the investigation (tensile strength analysis), the laser radiation was coupled into a 400 μm-core silica fiber. The diode was operated in continuous mode with a $1/e^2$ spot size at the protein solder surface of 1 mm. The laser system also incorporated a HeNe aiming beam, which was delivered through the same fiber as the 808 nm beam. Laser irradiances of 6.4, 12.7, 19.1, 25.5, and 31.8 W/cm^2, measured with a power meter (Molectron EPM 2,000 e power meter with PM30 thermopile detector, Molectron Detector Inc., Portland, OR), were delivered to the protein solder surface. Exposure times of 40, 60, 80, 100, and 200 seconds were used for the liquid protein solder and exposure times of 20, 30, 40, 50, and 100 seconds were used for the solid protein solder.

In the second part of the investigation (hydration study), the laser radiation was focused through a series of optics onto the specimen, which was held at a fixed position as indicated by a plastic marker. The diode was operated in continuous mode with a $1/e^2$ spot size at the protein solder surface of 1.5 mm. Laser irradiances of 4.9, 6.6, 8.2, and 12.3 W/cm^2, measured with a power meter, were delivered to the protein solder surface. This represented a range of irradiances around the optimum determined in the first part of the investigation. A translation stage moved the tissue under the beam at a scanning speed of 0.3 mm/second. Eight continuous passes were used for the liquid protein solder and four continuous passes were used for the solid protein solder. The length of each pass was 3.0 mm, thus the exposure times for the liquid protein solder and solid protein solder were 40 seconds and 80 seconds, respectively.

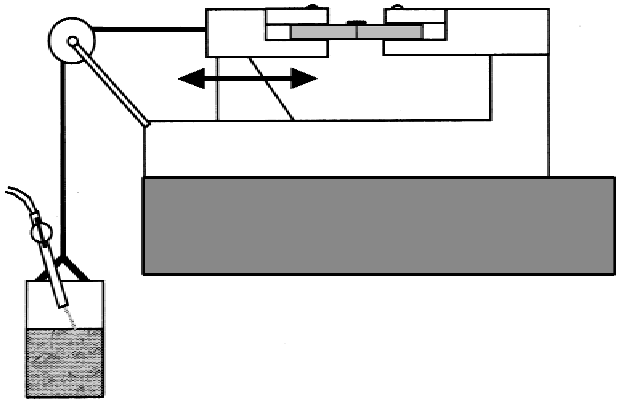


Fig. 2. Schematic of the gravity-based tensiometer for evaluating specimen tensile strength (after [37]).

Tensile Strength Analysis

Tensile strength measurements were performed to test the integrity of the resultant repairs immediately following the laser procedure using a gravity-based tensiometer [37]. The repaired specimen was mounted horizontally between metal grips of the tensiometer as shown in Figure 2. One grip was fixed and the other was allowed to translate on a sliding stage. The sliding grip was connected to a plastic container. Water was allowed to flow into the container at a constant rate of 450 ml/minute until the repair failed. Failure was defined as complete separation of the two halves of the specimen. The volume of water required to disrupt the repair was measured and then converted into a mass using the known density of water. The mass was divided by the cross-sectional area of the solder to yield tensile strength. Friction caused by the sliding stage and the weight of the plastic container were predetermined and subtracted from subsequent ten-

sile strength measurements. The specimens were kept moist during this procedure, to avoid tensile strength changes associated with drying [40]. The tensile strength of native aorta was also tested as a reference using specimens cut into dumbbell shapes with central width approximately the same width as the protein solders. Ten repairs were performed and tested for the four variations of protein solder at each of the five laser irradiances and exposure times investigated. Thus, a total of 1,000 tissue repairs were performed and tested for this analysis.

Hydration Study

The effect of hydration on bond stability was studied using both tensile strength analysis and scanning electron microscopy. Immediately following the laser procedure, specimens requiring tensile strength analysis were soaked in phosphate buffered saline (PBS) for a designated hydration period (1 hour, 1 day, 2 days, 1 week) then tested to determine the tensile strength of the repair. Acute tensile strength measurements were also made on a control group. Five repairs were performed and tested for the four variations of protein solder at each of the four laser irradiances and five hydration periods (including control) investigated. Thus, a total of 400 tissue repairs were performed in this study. The remaining specimens were prepared for SEM analysis. Specimens were re-cut, using a scalpel, along the line of the original tissue cut, to expose the solder/tissue cross-section. One half of each specimen was fixed immediately in 2.5% glutaraldehyde as a control and the other was soaked in PBS for the designated hydration period. After each hydration period, all specimens were removed from the PBS, fixed in glutaraldehyde, and prepared for SEM analysis. SEM analysis was performed on 40 specimens using a Jeol JSM 840 (Jeol Datum, Tokyo, Japan) scanning electron microscope.

RESULTS

Tensile Strength Analysis

Results of tensile strength measurements made on successfully repaired tissue specimens as a function of laser irradiance and exposure time are presented in Figures 3 and 4. The results of experiments conducted on liquid protein solder (25% BSA) at the two dye concentrations investigated, 2.5 mg/ml and 0.25 mg/ml ICG, are presented in Figure 3a and b, respectively, and the

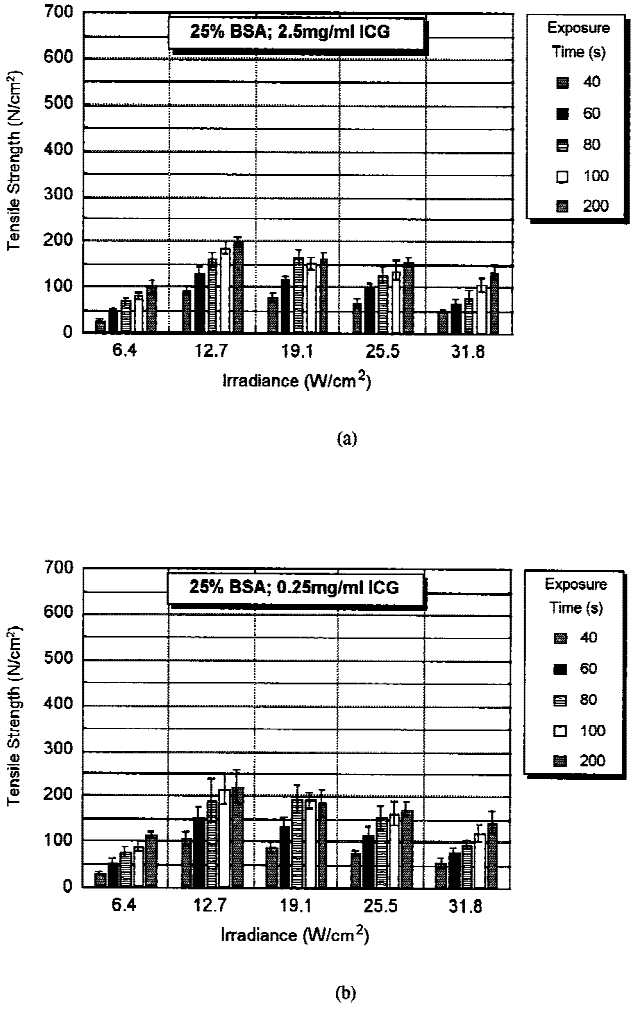


Fig. 3. Tensile strength of repairs formed using liquid protein solder (25% BSA) containing (**a**) 2.5mg/ml ICG; and (**b**) 0.25 mg/ml ICG, as a function of laser irradiance. Each graph shows results from the five different exposure times given in the legends. Each bar shows the mean and standard deviation for 10 repairs.

results of experiments conducted on solid protein solder (60% BSA) at the two dye concentrations investigated, are presented in Figure 4a and b, respectively. The tensile strength for each value of laser irradiance and exposure time was determined from the mean for 10 repairs. The standard deviation is also shown in each case. Repairs that failed to sustain a tensile strength above 10 N/cm^2 were deemed to be unsuccessful. Using the higher ICG dye concentration (2.5 mg/ml), 16 of the 266 liquid protein solder repairs and 2 of the 252 solid protein solder repairs tested fell into the unsuccessful category. Using the lower ICG dye concentration (0.25 mg/ml), 5 of the 255 liquid protein solder repairs and 0 of the 250 solid protein solder

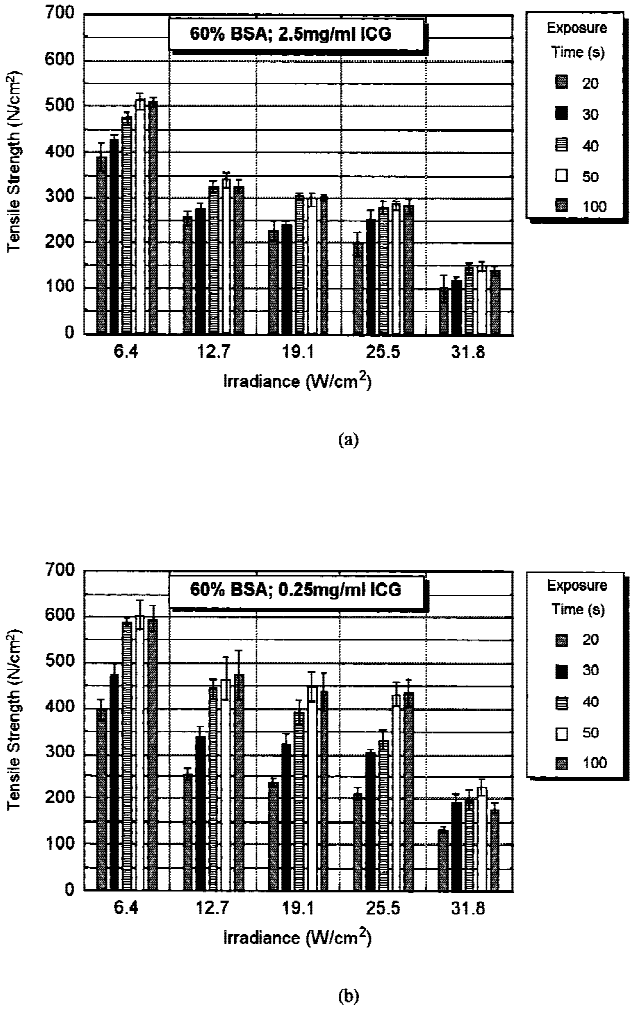


Fig. 4. Tensile strength of repairs formed using solid protein solder (60% BSA) containing (**a**) 2.5 mg/ml ICG; and (**b**) 0.25 mg/ml ICG, as a function of laser irradiance. Each graph shows results from the five different exposure times given in the legends. Each bar shows the mean and standard deviation for 10 repairs.

repairs tested were unsuccessful. This represented dehiscence rates of 6.0%, 0.8%, 2.0%, and 0%, respectively.

The liquid and solid protein solders exhibited two different mechanisms of failure. The liquid protein solder broke into two halves, but each remained attached to the tissue. The solid protein solder, however, remained intact but detached from the tissue. Nevertheless, the results in Figures 3 and 4 are presented in terms of the cross-section tensile strength for both the liquid and the solid solder. As shown in the graphs, the overall pull apart strength of repairs formed using the solid protein solder were significantly higher than the strength of the liquid protein solder repairs.

Several trends are evident from the graphs: (i) for the parameters investigated, tensile strength of the resulting repairs decreased significantly with increased irradiance except for the 6.4 W/cm^2 irradiance of the liquid solders; (ii) an exposure time existed after which further irradiation of the solid solder (60% BSA) did not improve the tensile strength of the repair; (iii) increasing BSA concentration from 25 to 60% greatly improved the tensile strength of the repairs; (iv) reducing ICG dye concentration from 2.5 mg/ml to 0.25 mg/ml led to repairs with higher tensile strengths.

Using the liquid protein solder with 2.5 mg/ml ICG (Fig. 3a), tensile strength doubled from a maximum of 99 ± 15 N/cm^2 to a maximum of 198 ± 11 N/cm^2 when irradiance doubled from 6.4 W/cm^2 to 12.7 W/cm^2. Beyond an irradiance of 12.7 W/cm^2, tensile strength slowly decreased. Lower ICG dye concentration (0.25 mg/ml ICG) showed a similar trend (Fig. 3b). Tensile strength increased by an average of 12% with the lower dye concentration over the range of irradiances investigated.

By using the solid protein solder with 2.5 mg/ml ICG (Fig. 4a), tensile strength decreased by approximately 70% from a maximum of 510 ± 17 N/cm^2 to a maximum of 148 ± 11 N/cm^2 when irradiance increased from 6.4 W/cm^2 to 31.8 W/cm^2. When ICG dye concentration was reduced to 0.25 mg/ml (Fig. 4b), tensile strength increased by an average of 44%. (The results of the hydration study using irradiances as low as 4.9 W/cm^2 confirm an irradiance of 6.4 W/cm^2 as being optimum, in terms of tensile strength, for repairs formed with the solid protein solder.)

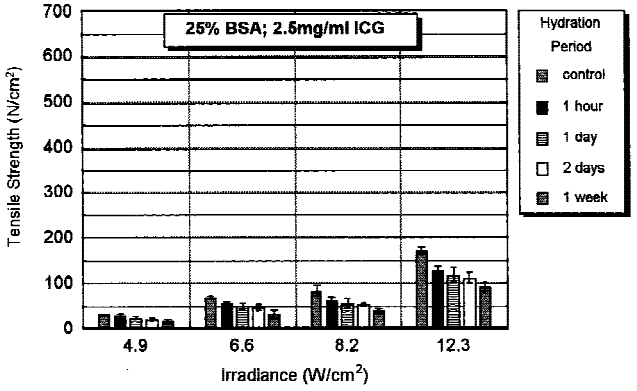
There was an optimum exposure time for the solid protein solder where further irradiation did not improve the tensile strength of the bond as illustrated in Figure 4a and b. An optimum exposure time was not always observed for the liquid solder. The liquid solder required much longer times to achieve coagulation (see Fig. 3a and b). This was most likely due to two effects: (i) the latent heat required during vaporization of water in the solder, and (ii) the higher thermal conductivity of the liquid solder causing the heat to spread out through the solder (unpublished data). Investigation of longer periods of irradiation should determine an optimum exposure time for the liquid protein solder.

The lower dye concentration of 0.25 mg/ml ICG gave better results for both liquid and solid solders. Irradiance and exposure time for optimal

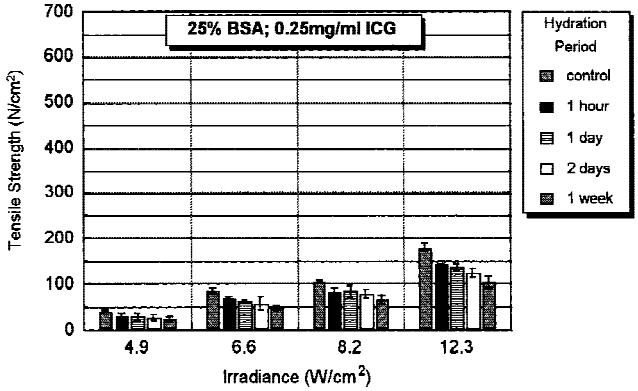
TABLE 1. Summary of Optimized Results From Tensile Strength Analysis. The Tensile Strength of Native Aorta Was Determined to be 596 ± 31 N/cm^2

Solder	Maximum tensile strength (N/cm^2)	Laser irradiance (W/cm^2)	Exposure time (seconds)
25% BSA; 2.5 mg/ml ICG	198 ± 11	12.7	100
25% BSA; 0.25 mg/ml ICG	220 ± 35	12.7	100
60% BSA; 2.5 mg/ml ICG	510 ± 17	6.4	50
60% BSA; 0.25 mg/ml ICG	602 ± 32	6.4	50

strength were determined from these results to be 12.7 W/cm^2 for 100 seconds with the liquid protein solder repairs and 6.4 W/cm^2 for 50 seconds with the solid protein solder repairs. The tensile strength of native aorta was measured to be 596 ± 31 N/cm^2. The maximum tensile strength achieved for solid protein solder repairs (642 ± 32 N/cm^2) was found to be comparable to this. The maximum tensile strength achieved for liquid protein solder repairs (220 ± 35 N/cm^2), however, was significantly inferior to native aorta and the solid solder repairs. Table 1 summarizes the results of the tensile strength analysis performed in this investigation.



(a)



(b)

Fig. 5. Tensile strength of repairs formed using liquid protein solder (25% BSA) containing (**a**) 2.5 mg/ml ICG; and (**b**) 0.25 mg/ml ICG, as a function of laser irradiance. Results are shown for the control and after soaking in PBS for designated hydration periods of 1 hour, 1 day, 2 days, or 1 week. Each bar shows the mean and standard deviation for five repairs.

Hydration Study

Tensile strength measurements. Results of tensile strength measurements made on repaired tissue specimens as a function of irradiance after various periods of hydration are presented in Figures 5 and 6. The results of experiments conducted using the liquid protein solder (25% BSA) containing 2.5 mg/ml and 0.25 mg/ml ICG, are presented in Figure 5a and b, respectively. The results of experiments conducted by using the solid protein solder (60% BSA) containing 2.5 mg/ml and 0.25 mg/ml ICG are presented in Figure 6a and b, respectively. The tensile strength for each value of laser irradiance and each hydration period has been determined from the mean for five repairs. The standard deviation is also shown.

Tensile strength decreased significantly (~20%) after the first hour of hydration with both the liquid and the solid protein solder. Using solders containing 2.5 mg/ml ICG, tensile strength decreased by approximately 25% after 1 hour hydration (see Figs. 5a and 6a). The tensile strength of the liquid solder repairs (Fig. 5a) slowly reduced with further hydration up to 1 week (20 to 50% change), whereas no significant change (student's t-test: $P < 0.05$) occurred to the tensile strength of the solid solder repairs (< 5% change) (Fig. 6a).

Similar results were found when the ICG dye concentration of the protein solders was reduced

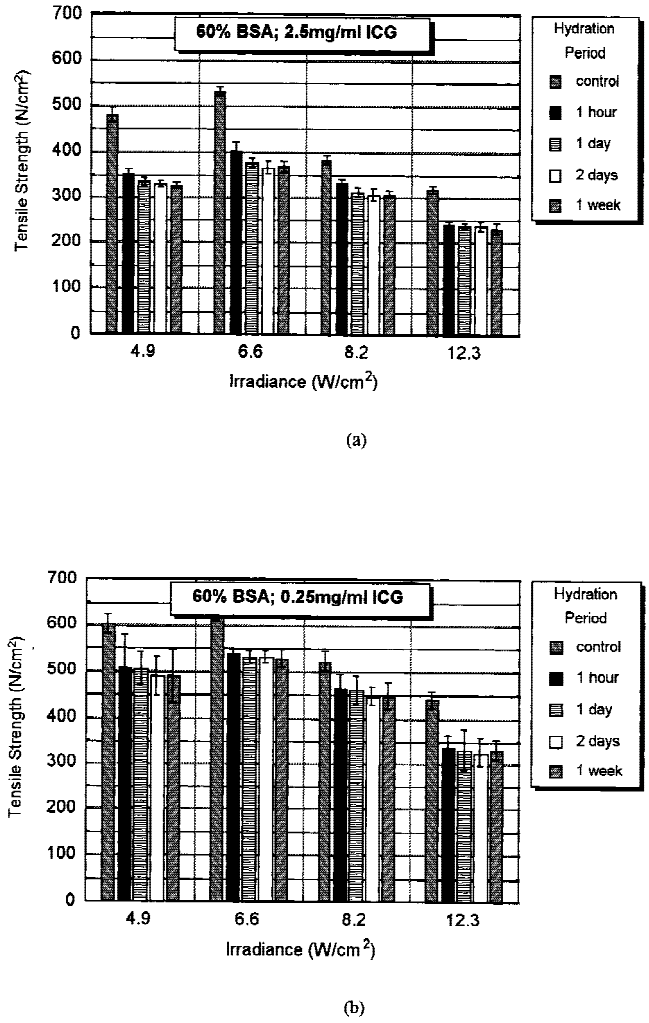


Fig. 6. Tensile strength of repairs formed using solid protein solder (60% BSA) containing (**a**) 2.5 mg/ml ICG; and (**b**) 0.25 mg/ml ICG, as a function of laser irradiance. Results are shown for the control and after soaking in PBS for designated hydration periods of 1 hour, 1 day, 2 days, or 1 week. Each bar shows the mean and standard deviation for five repairs.

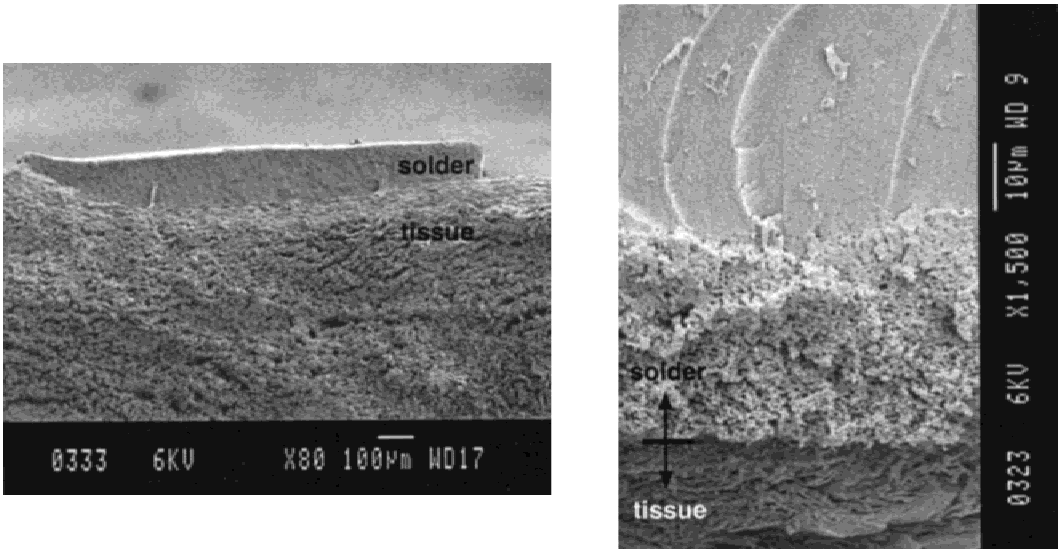
to 0.25 mg/ml (Figs. 5b and 6b). Tensile strength decreased by approximately 20% after 1 hour hydration. No appreciable change occurred to the tensile strength of the tissue repairs formed using solid protein solder with further hydration up to 1 week (Fig. 6b). The tensile strength of the liquid solder repairs (Fig. 5b), however, slowly reduced with further hydration.

Scanning electron microscopy analysis. Scanning electron micrographs of six of the treated specimens are shown in Figure 7. SEM shows the liquid protein solder method to suffer solder detachment after only 1 hour of hydration (Fig. 7c). On the other hand, using the solid solder, the solder coagulum remained well adhered to the tissue substrate even after 1 week of hydration (Fig. 7f). Non-uniform coagulation across the thickness of repairs formed using the liquid protein solder (Fig. 7a) is thought to be the cause of the significantly lower tensile strength (Fig. 3 vs. 4) and reduced stability during hydration (Fig. 5 vs. 6) of repairs formed by using liquid solders compared with solid protein solders.

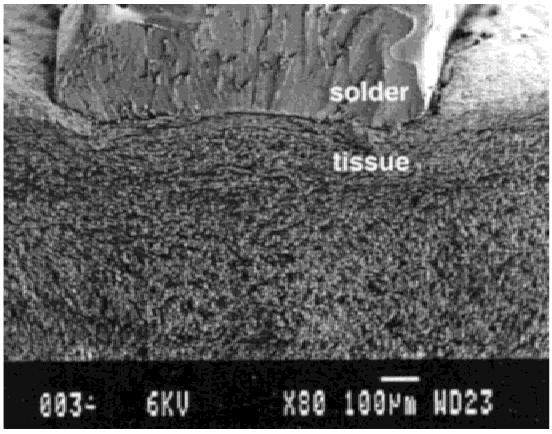
DISCUSSION

Previous studies have demonstrated that the addition of protein solders to augment laser tissue repairs significantly improves postoperative results [24,28,32,37,43]. This investigation goes further with the optimization of laser tissue soldering parameters.

The results of this investigation suggest that the strongest repairs are produced with lower irradiances of around 6.4 W/cm^2 after approximately 50 seconds exposure time by using a solid protein solder composed of 60% BSA and 0.25 mg/ml ICG. The most notable advantage of solid protein solder strips compared with other methods of laser-assisted tissue repair is the relatively high tensile strength of the resulting anastomosis. The maximum tensile strength of bonds formed by using the solid protein solder and 0.25 mg/ml ICG was 602 ± 32 N/cm^2 compared with 220 ± 35 N/cm^2 (0.25 mg/ml ICG) for liquid protein solder. More importantly, the tensile strength of repairs formed using the solid protein solder was comparable to that of native aorta (596 ± 31 N/cm^2). As discussed above, the principle risk of laser tissue welding is low tensile strength and the resulting high rate of dehiscence. Dehiscence rates of only 2.0% and 0% were experienced in this study using the optimized parameters for the liquid and solid protein solders, respectively. Liquid protein solders containing albumin concentrations greater than 50% have been found to be too viscous for easy handling and application to the tissue edges [39,43]. The solid protein solder strips, however, were easier to handle and apply to the tissue surface than the liquid protein solder. The slight rehydration of the solid solder surface during application improves adhesion so as to hold the tissue in close approximation during the repair procedure. This eliminates many of the problems associated with tissue apposition. A watertight closure can be achieved by placing the solid solder strips in close proximity to each other. In addition, the uniform dimensions of the solid protein solder strips allows pre-selection of optimal irra-



(a) liquid solder - control

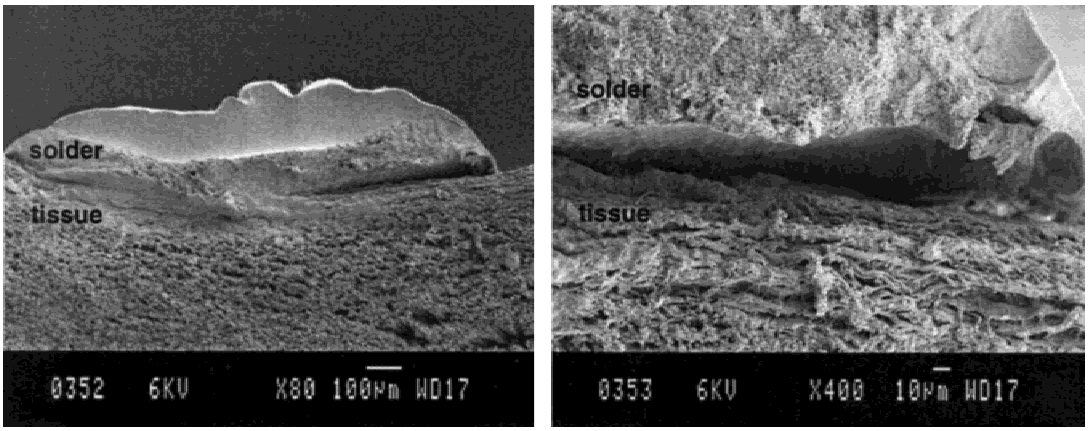


(b) solid solder - control

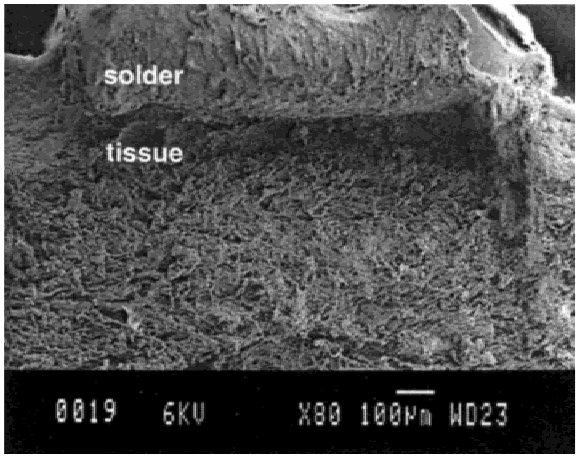
Fig. 7. Cross-section of tissue repairs formed using solders containing 0.25 mg/ml ICG after various periods of hydration: (**a**) liquid protein solder (25% BSA) control; (**b**) solid protein solder (60% BSA) control; (**c**) liquid protein solder after 1 hour of hydration; (**d**) solid protein solder after 1 hour of hydration; (**e**) liquid protein solder after 1 week of hydration; and (**f**) solid protein solder after 1 week of hydration. Liquid and solid solders were denatured using the optimized laser irradiances of 12.3 W/cm^2 and 6.6 W/cm^2, respectively.

diation parameters, hence reducing the "end-point" ambiguity of the procedure.

Finally, repairs formed with the solid protein solder are more stable than repairs formed with the liquid protein solder under hydration. SEM showed the liquid protein solder to suffer solder detachment after only 1 hour of hydration. This observation was consistent with the findings of Chan et al. where an 808 nm CW laser beam with an irradiance of 27 W/cm^2 and an ICG-doped albumin solder (0.25 mg/ml ICG, 25% human albumin, and 0.5% sodium hyaluronate) was used to repair rat dermis and baboon articular cartilage in vitro [37,39]. Dehiscence was noted in some of the specimens as early as 1 hour after hydration. SEM analysis showed the solder to be densely coagulated in the upper solder region close to the laser beam whilst the solder in the region close to the solder/tissue interface had aggregated into protein globules. The protein globules formed



(c) liquid solder - after 1 day of hydration



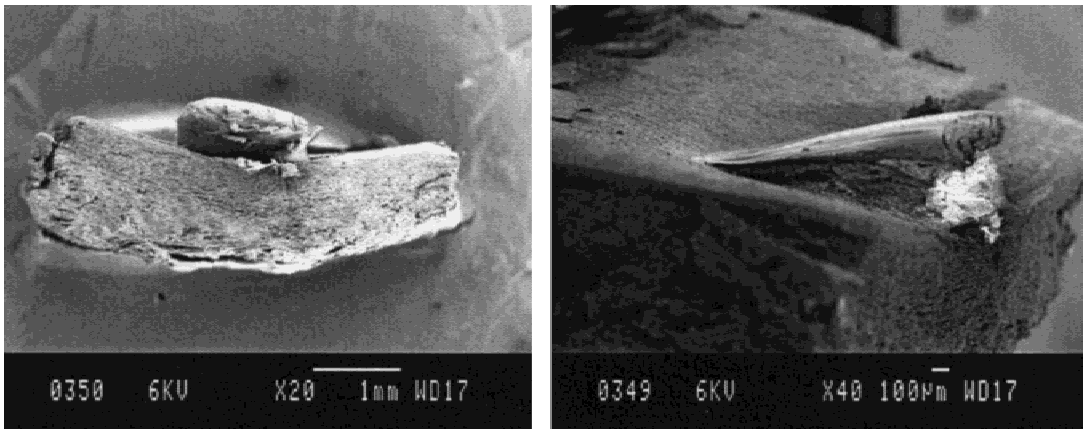
(d) solid solder - after 1 day of hydration

Fig. 7. Continued.

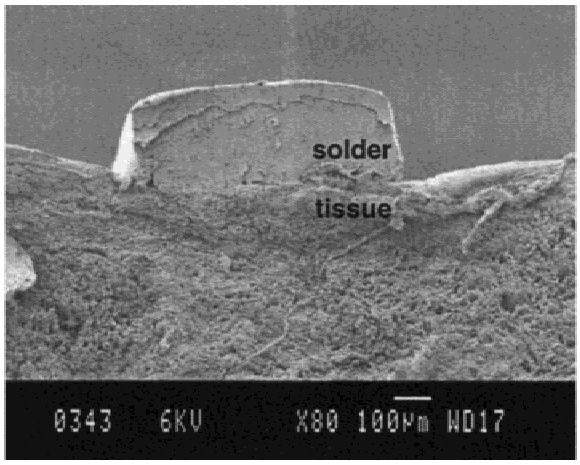
weak bonds with the tissue proteins, so they were easily disrupted when submerged in a hydrated environment.

The solid protein solder of this study, however, remained well adhered to the tissue substrate even after 1 week of hydration. Although tensile strength was observed to decrease significantly (20 to 25%) after the first hour of hydration for both the liquid and the solid protein solders, it only continued to decrease in the liquid protein solder with further hydration confirming that weaker solder/tissue bonds were formed using the liquid protein solder. As the collagen-albumin bonding reactions achieved higher density as the concentration of the protein increased [44], these results are not unexpected. Also of interest was the fact that in a recent study by Chan et al., the strength of liquid solder repairs was improved by using a series of small droplets and fully coagulating each droplet [41,42].

This was an in vitro study, and thus, factors that influence tissue regeneration were not taken into account, such as the effects of the laser and the solder on surrounding tissue, wound healing, neovascularization, and tension on the repair site. In particular, the in vitro analysis of tensile strength and dehiscence rates following hydration, in the absence of wound healing, may be unrelated to the in vivo condition. Finally, the addition of other components to the solder has not been investigated though they can significantly affect the outcome. Hyaluronate, a nonpyrogenic,



(e) liquid solder - after 1 week of hydration



(f) solid solder - after 1 week of hydration

Fig. 7. Continued.

viscoelastic preparation of highly purified, noninflammatory substance found in the extracellular matrix of connective tissues in humans and animals, is commonly used in albumin solder compositions and has been found to significantly increase tensile strength of resulting repairs [6]. The incorporation of human thrombin into human albumin tissue solders is a safe and effective means of reducing bleeding [45]. Using growth factors such as TGF-β_1 to enhance albumin solder increases the strength of laser repaired wounds and provides a means to accelerate wound healing [17]. Other possible additives include anti-inflammatory agents, angiogenic agents, and antibiotics [6]. These additional factors are worthy of further investigation.

CONCLUSIONS

Research into the use of laser-activated protein solders continues to promote the technique of laser tissue soldering as a useful adjunct to tissue repair. Careful in vitro studies of laser tissue repair with protein solders were needed to improve the procedure. In this study of over 1,400 tissue repairs, using protein solders composed of bovine serum albumin and indocyanine green dye, optimal parameters including irradiance and exposure time have been identified based on criteria such as the immediate tensile strength of the repairs and determination of the effect of hydration on the resultant repairs. Tensile strength was found to be improved with the use of increased

protein concentrations and lower dye concentrations in the protein solder, and the optimal laser irradiance was reduced for higher concentration protein solders. The postoperative success of laser tissue repairs is significantly influenced by the strength of the repair under hydration. The solid protein solder provided more stable adhesion to the tissue than did the liquid protein solder when the tissue was submerged in a hydrated environment. Although the results obtained in this study are very encouraging for the application of laser activated protein solders for tissue repair, further in vivo studies of postoperative recovery rate are needed using the optimal parameters we have determined, to establish their clinical suitability.

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